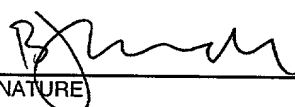


FORM PTO-1390 (REV 11-2000)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER <b>3557-11</b>
<b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b>		U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) <b>10/088027</b> <small>Unknown</small>
INTERNATIONAL APPLICATION NO. <b>PCT/EP00/09245</b>	INTERNATIONAL FILING DATE <b>21 September 2000</b>	PRIORITY DATE CLAIMED <b>1 October 1999</b>
TITLE OF INVENTION <b>GMP SYNTHETASE DERIVED FROM PLANTS</b>		
APPLICANT(S) FOR DO/EO/US <b>LERCHL et al</b>		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.</li> <li>4. <input checked="" type="checkbox"/> The U.S. has been elected by the expiration of 19 months from the priority date (Article 31).</li> <li>5. A copy of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is attached hereto.</li> <li>b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).</li> </ol> </li> <li>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input type="checkbox"/> have been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has <b>NOT</b> expired.</li> <li>d. <input type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</li> <li>10. <input checked="" type="checkbox"/> A English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol>		
<b>Items 11 To 20 below concern document(s) or information included:</b>		
<ol style="list-style-type: none"> <li>11. <input type="checkbox"/> An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.</li> <li>12. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.</li> <li>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.</li> <li>14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</li> <li>15. <input type="checkbox"/> A substitute specification.</li> <li>16. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>17. <input checked="" type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.</li> <li>18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</li> <li>19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</li> <li>20. <input checked="" type="checkbox"/> Other items or information. PTO-1449 and copy of International Search Report and Statement with paper and computer readable copies of Sequence Listing</li> </ol>		

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) <b>104088027</b>		INTERNATIONAL APPLICATION NO. <b>PCT/EP00/09245</b>		ATTORNEY'S DOCKET NUMBER <b>3557-11</b>	
21. <input checked="" type="checkbox"/> The following fees are submitted:				<b>CALCULATIONS</b> PTO USE ONLY	
<b>BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5):</b> -- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .....\$1040.00 -- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .....\$890.00 -- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO .....\$740.00 -- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .....\$710.00 -- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) .....\$100.00  <div style="text-align: right;"><b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b></div>				<div style="text-align: right;">\$ 890.00</div>	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)). <div style="text-align: right;">\$ 0.00</div>					
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	25	-20 =	5	X	\$18.00
Independent Claims	5	-3 =	2	X	\$84.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)				\$280.00	\$ 280.00
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$	<b>1428.00</b>
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				0.00	
<b>SUBTOTAL =</b>				\$	<b>1428.00</b>
Processing fee of \$130.00, for furnishing the English Translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)).				+	0.00
<b>TOTAL NATIONAL FEE =</b>				\$	<b>1428.00</b>
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). <b>\$40.00</b> per property				+	\$ 40.00
Fee for Petition to Revive Unintentionally Abandoned Application (\$1280.00 - Small Entity = \$640.00)				\$	0.00
<b>TOTAL FEES ENCLOSED =</b>				\$	<b>1468.00</b>
				Amount to be:	
				refunded	\$
				Charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$1468.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. 14-1140 in the amount of \$_____ to cover the above fees. A duplicate copy of this form is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed. d. <input checked="" type="checkbox"/> The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.					
<b>NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>					
<b>SEND ALL CORRESPONDENCE TO:</b>  NIXON & VANDERHYE P.C. 1100 North Glebe Road, 8 <sup>th</sup> Floor Arlington, Virginia 22201-4714 Telephone: (703) 816-4000					
				 SIGNATURE	
				<b>B. J. Sadoff</b> NAME	
				<b>36,663</b> REGISTRATION NUMBER	
				<b>March 14, 2002</b> Date	

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

**LERCHL et al**

Atty. Ref.: **3557-11**

Serial No. **Unknown**

Group:

National Phase of: **PCT/EP00/09245**

International Filing Date: **21 September 2000**

Filed: **March 14, 2002**

Examiner:

For: **GMP SYNTHETASE DERIVED FROM PLANTS**

\* \* \* \* \*

**March 14, 2002**

Assistant Commissioner for Patents  
Washington, DC 20231

Sir:

**PRELIMINARY AMENDMENT**

Prior to calculation of the filing fee and in order to place the above identified application in better condition for examination, please amend as follows:

**IN THE SPECIFICATION**

Page 1, after the title insert the following:

-- This application is the US national phase of international application

PCT/EP00/09245 filed September 21, 2000 which designated the U.S. --.

Insert the attached Sequence Listing in place of the originally-filed Sequence Listing.

**IN THE CLAIMS**

Please substitute the following amended claims for corresponding claims previously presented. A copy of the amended claims showing current revisions is attached.

14. (Amended) An inhibitor of plant GMP synthetase identified using an assay system as claimed in claim 11.

10088027-031402

LERCHL et al  
Serial No. **Unknown**

15. (Amended) An inhibitor as claimed in claim 13 for use as herbicide.

Please add the following new claims:

17. (New) An inhibitor of plant GMP synthetase identified using an assay system as claimed in claim 12.

18. (New) An inhibitor as claimed in claim 14 for use as herbicide.

### **REMARKS**

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

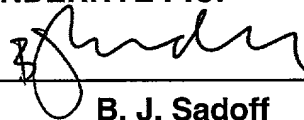
The attached paper and computer readable copies of the Sequence Listing are the same. No new matter has been added. A separate Statement to this effect is attached.

The above amendments are made to place the claims in a more traditional format.

Respectfully submitted,

**NIXON & VANDERHYE P.C.**

By: \_\_\_\_\_



**B. J. Sadoff**

Reg. No. 36,663

**BJS:Imy**

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

14. (Amended) An inhibitor of plant GMP synthetase identified using an assay system as claimed in claim 11 [or 12].

15. (Amended) An inhibitor as claimed in [either of claims 13 or 14] claim 13 for use as herbicide.

u/ppts

## GMP synthetase from plants

The present invention relates to the identification of plant GMP  
5 synthetase (guanosine-monophosphate synthetase) as novel target  
for herbicidal agents. The present invention further relates to  
DNA sequences coding for a polypeptide having GMP synthetase  
(EC 6.3.5.2) activity. The invention moreover relates to the use  
of a nucleic acid coding for a protein having GMP synthetase  
10 activity of plant origin for producing an assay system for  
identifying inhibitors of GMP synthetase having a herbicidal  
action, and to inhibitors of plant GMP synthetase identified  
using this assay system. The invention further relates to the use  
of the nucleic acid coding for plant GMP synthetase for producing  
15 plants with increased resistance to inhibitors of GMP synthetase,  
and for producing plants with a modified content of guanosine  
nucleotides. The invention additionally relates to a method for  
eliminating unwanted plant growth, which comprises treating the  
plants to be eliminated with a compound which specifically binds  
20 to GMP synthetase encoded by a DNA sequence SEQ-ID No. 1 or a DNA  
sequence hybridizing with the latter, and inhibits the function  
thereof.

Plants are able to synthesize their cellular components from  
25 carbon dioxide, water and inorganic salts.

This process is possible only through the use of biochemical  
reactions to synthesize organic substances. It is necessary for  
plants to synthesize de novo the nucleotides as constituents of  
30 nucleic acids.

Especially in rapidly growing plant tissues it is necessary for  
nucleotides as constituents of the nucleic acids DNA and RNA to  
be synthesized by multistage metabolic pathways. Nucleotides are  
35 moreover linked in with virtually all metabolic pathways.  
Nucleoside triphosphates, especially ATP, drive many  
energy-expendng reactions in cells. Adenine nucleotide  
additionally occurs as component in essential coenzymes such as  
coenzyme A and nicotinamide and flavin coenzymes, which are  
40 involved in many cellular conversions. Guanosine nucleotides give  
a reaction direction to various cellular processes such as  
protein translation, microtubule assembly, vesicular transport,  
signal transduction and cell division. In addition, nucleotides  
are the starting metabolites for the biosynthesis of  
45 methylxanthines such as caffeine and theobromine, especially in  
the Rubiaceae and Theaceae families of plants.

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Purine nucleotides are formed in microorganisms, animals and plants de novo in the same way starting from phosphoribosyl pyrophosphate (PRPP). IMP is synthesized in a 10-stage reaction sequence. IMP can be converted in subsequent reactions by  
5 adenylosuccinate synthetase and adenylosuccinate lyase into AMP. In the synthesis of GMP there is initial conversion of IMP by IMP dehydrogenase into XMP which is aminated by GMP synthetase to give GMP, see Fig. 1.

- 10 Genes which code for GMP synthetase [sic] have been isolated from various organisms.

The compartmentation of the purine biosynthetic pathway in plants has not to date been extensively investigated. The nitrogen which  
15 is fixed in the form of glutamine and aspartate in the root nodules of legumes is firstly converted via the de novo synthetic pathway into purines. This pathway is localized in the plastids in the root nodules of *Glycine max* and *Vigna unguiculata* L. (Boland and Schubert, Arch. Biochem. Biophys. 220 (1983),  
20 179-187; Shelp et al., Arch. Biochem. Biophys. 224 (1983), 429-441). However, more recent investigations have shown that enzyme activities of the purine biosynthetic pathway are also to be found in mitochondria in the root nodules of *Vigna unguiculata* [sic] (Atkins et al., Plant Physiology 113 (1997), 127-135; Smith  
25 et al., Plant Molecular Biology 36 (1998), 811-820).

The regulation of this synthetic pathway has to date been investigated only in microorganisms and animals and comprises transcription control, end-product inhibition and allosteric  
30 regulation. The enzyme PRPP amidotransferase (PRPP ATase) of the second reaction step is attributed with a key position in the animal as well as the plant system and is subject to allosteric regulation by the end products IMP, AMP and GMP (Reynolds et al., Archives of Biochemistry and Biophysics 229 (1984), 623-631).

35 GMP-Synthetase also plays a part in relation to the balanced synthesis of guanosine nucleotides and adenosine nucleotides because ATP is a substrate of GMP synthetase.

40 Since plants are dependent on a functioning nucleotide metabolism, this metabolism is obviously a possible target of novel herbicides. In fact, agents with an inhibitory effect on enzymes of de novo purine biosynthesis have already been described. An example which may be mentioned is  
45 5'-phosphohydantocidin which inhibits an enzyme of plant purine metabolism, adenylosuccinate synthetase (ASS) (Siehl et al., Plant Physiol. 110 (1996), 753-758). Inhibitors for enzymes of

10088027-034402

this metabolic pathway also exist from animals and microorganisms. Folate analogs inhibit various folate-dependent reactions, inter alia the enzyme GAR transformylase and have antiproliferative, antiinflammatory and immunosuppressant effects. Mycophenolate (MPA), as an inhibitor of IMP dehydrogenase, has antimicrobial, antiviral and immunosuppressant effects. (Kitchin et al., Journal of the American Academy of Dermatology 37 (1997), 445-449).

- 10 Demonstration of the suitability of an enzyme as herbicide target can be shown, for example, by reducing the enzyme activity by means of the antisense technique in transgenic plants. If reduced growth is brought about in this way, it can be concluded that the enzyme whose activity has been reduced is a suitable site of action of herbicidal agents. This has been shown by way of example for acetolactate synthase on transgenic potato plants (Höfgen et al., Plant Physiology 107 (1995), 469-477).

- It is an object of the present invention to prove that GMP synthetase in plants is a suitable herbicidal target, to isolate a complete plant cDNA coding for the enzyme GMP synthetase and functional expression thereof in bacterial or eukaryotic cells, and to produce an efficient and simple GMP synthetase assay system for carrying out the inhibitor-enzyme binding studies.

- 25 We have found that this object is achieved by isolation of a gene coding for the plant enzyme GMP synthetase, the production of antisense constructs of GMP synthetase, and functional expression of the GMP synthetase in bacterial or eukaryotic cells.

- 30 One aspect of the present invention relates to the isolation of a full-length cDNA coding for a functional glutamine-hydrolyzing GMP synthetase (EC 6.3.5.2.) from tobacco (*Nicotiana tabacum*).

- 35 A first aspect of the present invention is a DNA sequence SEQ-ID NO:1 comprising the coding region of a plant GMP synthetase from tobacco, see Example 1.

- Another aspect of the invention is a DNA sequence SEQ-ID No. 3 comprising a portion of the coding region of a plant GMP synthetase from *Physcomitrella patens*, see Example 2.

- Further aspects of the invention are DNA sequences which are derived from SEQ-ID NO: 1 or SEQ-ID No: 3 or hybridize with one of these sequences and code for a protein which has the biological activity of a GMP synthetase.

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Tobacco plants of the line *Nicotiana tabacum* cv. Samsun NN harboring an antisense construct of GMP synthetase have been characterized in detail. The plants show growth retardation to differing extents. The transgenic lines, and the progeny as 1<sup>st</sup> and 2<sup>nd</sup> generation showed reduced growth in soil. In plants with reduced growth it was possible to detect a reduced, compared with the wild-type, GMP-7M RNA amount in the Northern hybridization. It was also possible in a Western blot experiment to detect a reduced amount, compared with wild-type plants, of GMP synthetase in the transgenic lines, see Example 7. A correlation can be found in the growth retardation and reduction in the amount of GMP synthetase protein. This clear association demonstrates for the first time that GMP is unambiguously a suitable target protein for herbicidal agents.

In order to be able to find efficient inhibitors of plant GMP synthetase, it is necessary to provide suitable assay systems with which inhibitor-enzyme binding studies can be carried out. For this purpose, for example, the complete cDNA sequence of the GMP synthetase from tobacco is cloned in an expression vector (pQE, Qiagen) and is expressed in *E. coli*, see Example 4.

An alternative possibility is, however, to express the expression cassette comprising a DNA sequence SEQ-ID No. 1 for example in other bacteria, in yeasts, fungi, algae, plant cells, insect cells or mammalian cells, see Example 5.

The GMP synthetase protein expressed with the aid of the expression cassette according to the invention is particularly suitable for finding inhibitors specific for GMP synthetase.

For this purpose, the plant GMP synthetase can be employed, for example, in an enzyme assay in which the activity of the GMP synthetase is measured in the presence and absence of the agent to be tested. Qualitative and quantitative information about the inhibitory characteristics of the agent to be tested is obtainable from comparison of the two activity determinations, see Example 8.

The assay system according to the invention can be used for rapid and simple testing of a large number of chemical compounds for herbicidal properties. The method allows reproducible selection from a large number of substances specifically of those having a potent effect in order then to carry out other, more intensive tests which are familiar to the skilled worker on these substances.

A further aspect of the invention is a method for identifying substances having a herbicidal action, which inhibit the GMP synthetase activity in plants, consisting of

- 5 a) preparation of transgenic plants, plant tissues, or plant cells which comprise an additional DNA sequence coding for an enzyme having GMP synthetase activity and are able to overexpress an enzymatically active GMP synthetase;
- 10 b) application of a substance to transgenic plants, plant cells, plant tissues or plant parts and to untransformed plants, plant cells, plant tissues or plant parts;
- 15 c) determination of the growth or survivability of the transgenic and untransformed plants, plant cells, plant tissues or plant parts after application of the chemical substance; and
- 20 d) comparison of the growth or survivability of the transgenic and untransformed plants, plant cells, plant tissues or plant parts after application of the chemical substance;

where suppression of the growth or survivability of the untransformed plants, plant cells, plant tissues or plant parts

- 25 without, however, greatly suppressing the growth or the survivability of the transgenic plants, plant cells, plant tissues or plant parts demonstrates that the substance from b) shows herbicidal activity and inhibits the GMP synthetase enzymic activity in plants.

30

A further aspect of the invention is a method for identifying inhibitors of plant GMP synthetases, with potential herbicidal action, by cloning the gene of a plant GMP synthetase, bringing about overexpression in a suitable expression cassette - for

- 35 example in insect cells, opening the cells and employing the cell extract directly or after concentration or isolation of the enzyme GMP synthetases in an assay system for measuring the enzymic activity in the presence of low molecular weight chemical compounds.

40

A further aspect of the invention comprises compounds having a herbicidal action which can be identified using the assay system described above.

- 45 A further aspect of the invention is a method for eliminating unwanted plant growth, which comprises treating the plants to be eliminated with a compound which specifically binds to plant GMP

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synthetase and inhibits the function thereof.

Inhibitors of GMP synthetase with a herbicidal action can be used as defoliants, desiccants, haulm destroyers and, in particular, 5 weedkillers. Weeds mean in the widest sense all plants which grow where they are unwanted. Whether the agents found with the aid of the assay system according to the invention act as total or selective herbicides depends inter alia on the application rate.

- 10 Inhibitors of GMP synthetase with a herbicidal action can be used, for example, to control the following weeds:

Dicotyledonous weeds of the genera:

- Sinapis, Lepidium, Galium, Stellaria, Matricaria, Anthemis,  
15 Galinsoga, Chenopodium, Urtica, Senecio, Amaranthus, Portulaca, Xanthium, Convolvulus, Ipomoea, Polygonum, Sesbania, Ambrosia, Cirsium, Carduus, Sonchus, Solanum, Rorippa, Rotala, Lindernia, Lamium, Veronica, Abutilon, Emex, Datura, Viola, Galeopsis, Papaver, Centaurea, Trifolium, Ranunculus, Taraxacum.

20

Monocotyledonous weeds of the genera:

- Echinochloa, Setaria, Panicum, Digitaria, Phleum, Poa, Festuca, Eleusine, Brachiaria, Lolium, Bromus, Avena, Cyperus, Sorghum, Agropyron, Cynodon, Monochoria, Fimbristylis, Sagittaria,  
25 Eleocharis, Scirpus, Paspalum, Ischaemum, Sphenoclea, Dactyloctenium, Agrostis, Alopecurus, Apera.

Another aspect of the invention comprises expression cassettes whose sequence codes for a GMP synthetase from tobacco or

- 30 functional equivalent thereof. The nucleic acid sequence may in this connection be, for example, a DNA or a cDNA sequence.

Another aspect of the invention is an expression cassette comprising a DNA sequence SEQ-ID No. 3 coding for a portion of

- 35 the plant GMP synthetase from *Physcomitrella patens*.

The expression cassettes according to the invention additionally comprise regulatory nucleic acid sequences which control the expression of the coding sequence in the host cell. In a

- 40 preferred embodiment, an expression cassette according to the invention comprises a promoter upstream, i.e. at the 5' end of the coding sequence, and a polyadenylation signal downstream, i.e. at the 3' end, and, where appropriate, further regulatory elements which are operatively linked to the GMP synthetase gene  
45 coding sequence lying between them. An operative linkage means the sequential arrangement of promoter, coding sequence, terminator and, where appropriate, further regulatory elements in

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such a way that each of the regulatory elements is able to perform its function as intended on expression of the coding sequence.

- 5 An expression cassette according to the invention is produced by fusing a suitable promoter with a suitable GMP synthetase DNA sequence and a polyadenylation signal by conventional recombination and cloning techniques as described, for example, in J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989) and in T.J. Silhavy, M.L. Berman and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and in Ausubel, F.M. et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley-Interscience (1987).

- Another aspect of the invention comprises functionally equivalent DNA sequences which code for a GMP synthetase and which have, based on the complete length of the DNA sequence, a sequence  
20 homology with the DNA sequence SEQ-ID NO: 1 or SEQ-ID No. 3 of from 40 to 100%.

- A preferred aspect of the invention comprises functionally equivalent DNA sequences which code for a GMP synthetase and  
25 which have, based on the complete length of the DNA sequence, a sequence homology with the DNA sequence SEQ-ID NO: 1 or SEQ-ID No. 3 of from 60 to 100%.

- A particularly preferred aspect of the invention comprises  
30 functionally equivalent DNA sequences which code for a GMP synthetase and which have, based on the complete length of the DNA sequence, a sequence homology with the DNA sequence SEQ-ID NO: 1 or SEQ-ID No. 3 of from 80 to 100%.
- 35 Functionally equivalent sequences which code for a GMP synthetase are, according to the invention, sequences which, despite a different nucleotide sequence, still have the desired functions. Functional equivalents thus comprise naturally occurring variants of the sequences described herein, and artificial nucleotide  
40 sequences, for example obtained by chemical synthesis, which are adapted to the codon usage of a plant.

- A functional equivalent also means in particular natural or artificial mutations of an originally isolated sequence which  
45 codes for a GMP synthetase and additionally shows the required function. Mutations comprise substitutions, additions, deletions, transpositions or insertions of one or more nucleotide residues.

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Thus, for example, the present invention also includes nucleotide sequences obtained by modification of the nucleotide sequence. The aim of such a modification may be, for example, further localization of the coding sequence present therein or, for  
5 example, insertion of further restriction enzyme cleavage sites.

Functional equivalents are also variants whose function has been attenuated or enhanced by comparison with initial gene or gene fragment.

10

The expression cassette according to the invention can also be employed for transforming bacteria, cyanobacteria, yeasts, filamentous fungi and algae with the aim of producing adequate amounts of the enzyme GMP synthetase.

15

A further aspect of the invention is a protein from tobacco which has the amino acid sequence SEQ-ID NO: 2 or derivatives or portions of this protein with GMP synthetase activity.

20 Another aspect of the invention comprises plant proteins with GMP synthetase activity having an amino acid sequence homology with the tobacco GMP synthetase of 20 - 100% identity.

Preferred plant proteins with GMP synthetase activity have an  
25 amino acid sequence homology with the tobacco GMP synthetase of 50 - 100% identity.

Particularly preferred plant proteins with GMP synthetase activity have an amino acid sequence homology with the tobacco  
30 GMP synthetase of 80 - 100% identity.

Another aspect of the invention comprises plant proteins with GMP synthetase activity having an amino acid sequence homology with the *Physcomitrella patens* GMP synthetase of 20 - 100% identity.

35

Preferred plant proteins with GMP synthetase activity have an amino acid sequence homology with the *Physcomitrella patens* GMP synthetase of 50 - 100% identity.

40 Particularly preferred plant proteins with GMP synthetase activity have an amino acid sequence homology with the *Physcomitrella patens* GMP synthetase of 80 - 100% identity.

A further object of the invention was overexpression of the GMP  
45 synthetase gene in plants to produce plants which are tolerant of inhibitors of GMP synthetase.

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Overexpression of the gene sequence SEQ-ID NO: 1 coding for a GMP synthetase in a plant achieves increased resistance to inhibitors of GMP synthetase. The transgenic plants produced in this way are likewise an aspect of the invention.

5

The efficiency of expression of the transgenically expressed GMP synthetase gene can be measured, for example, in vitro by shoot meristem propagation or by a germination test. In addition, a change in nature and level of the expression of the GMP

- 10 synthetase gene and the effect thereof on the resistance to inhibitors of GMP synthetase can be tested on test plants in glasshouse experiments.

An additional aspect of the invention comprises transgenic plants

- 15 transformed with an expression cassette according to the invention comprising DNA sequence SEQ-ID No. 1, which has become tolerant of inhibitors of GMP synthetase due to additional expression of DNA sequence SEQ-ID No. 1, and to transgenic cells, tissues, parts and propagation material of such plants.

- 20 Particular preference is given in this connection to transgenic crop plants such as, for example, barley, wheat, rye, corn, soybean, rice, cotton, sugar beet, canola, sunflower, flax, hemp, potato, tobacco, tomato, oilseed rape, alfalfa, lettuce and the various tree, nut and vine species, and legumes.

25

An alteration in the nucleotide content in plants may be beneficial in various cases. For example, nucleotides are added to plant-based babyfood products in order to achieve a nutrient solution corresponding to breast milk. In addition, an optimized

- 30 nucleotide content would be sensible in the enteral feeding of patients. A reduced purine nucleotide content in plants of foods relevance is relevant to the dietary feeding of patients with gout. Nucleotides also have flavor-forming and flavor-enhancing effects so that an altered nucleotide content has effects on the  
35 taste properties of plants.

A further aspect of the invention therefore comprises plants which have a modified content of guanosine nucleotides after expression of the DNA sequence SEQ-ID NO: 1 or SEQ-ID No: 3 in

- 40 the plant.

A plant with a modified content of guanosine nucleotides is based, for example, by expression of an additional DNA sequence SEQ-ID No. 1 or 3 in the sense or antisense orientation in the

- 45 plant. A modified content of guanosine nucleotides means that it is possible to produce plants with an increased content of guanosine nucleotides in the case of the sense orientation and

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plants with a reduced content of guanosine nucleotides in the case of the sense orientation (cosuppression) or antisense orientation.

- 5 Increasing the content of guanosine nucleotides means, for example, within the scope of the present invention the artificially applied capability of increased biosynthesis of guanosine nucleotides owing to functional overexpression of the GMP synthetase gene in the plant compared with the plant which  
10 has not been genetically manipulated for the duration of at least one plant generation.

A further aspect of the invention is the use of plant GMP synthetases to alter the concentrations of methylxanthines in  
15 plants.

- Particularly preferred sequences are those which ensure targeting in the apoplasts, in plastids, the vacuoles, the mitochondrion, the endoplasmic reticulum (ER) or, through the absence of  
20 appropriate operative sequences, ensure retention in the compartment of production, the cytosol (Kermode, Crit. Rev. Plant Sci. 15, 4 (1996), 285-423).

- For example, the plant expression cassette can be incorporated  
25 into the tobacco transformation vector pBinAR, see Example 6.

- Suitable in principle as promoter for the expression cassette according to the invention is every promoter able to control expression of foreign genes in plants. It is particularly  
30 preferred to use a plant promoter or a promoter derived from a plant virus. The CaMV 35S promoter from Blumenkohl mosaic virus (Franck et al., Cell 21 (1980), 285-294) is particularly preferred. This promoter contains various recognition sequences for transcriptional effectors which, in their totality, lead to  
35 permanent and constitutive expression of the introduced gene (Benfey et al., EMBO J. 8 (1989), 2195-2202).

- The expression cassette according to the invention may also comprise a chemically inducible promoter through which it is  
40 possible to control expression of the exogenous GMP synthetase gene in the plant at a particular point in time. Promoters of this type, such as, for example, PRP1 promoter (Ward et al., Plant. Mol. Biol. (1993) 22, 361-366), a promoter inducible by salicylic acid (WO 95/19443), a benzenesulfonamide-inducible  
45 (EP 388186), a tetracycline-inducible (Gatz et al., Plant J. (1992) 2, 397-404), an abscisic acid-inducible (EP0335528) or an ethanol- or cyclohexanone-inducible (WO 93/21334) promoter are

described in the literature and can, inter alia, be used.

Further particularly preferred promoters are those which ensure expression in tissues or plant parts in which the biosynthesis of purines or their precursors takes place. Particular mention may be made of promoters which ensure leaf-specific expression. Mention should also be made of the promoter of the potato cytosolic FBPase or the potato ST-LSI promoter (Stockhaus et al., EMBO J., (1989) 8, 2445-2451).

10

It is possible with the aid of a seed-specific promoter to express a foreign protein stably up to a content of 0.67% of the total soluble seed protein in the seed of transgenic tobacco plants (Fiedler and Conrad, Bio/Technology (1995) 10, 1090-1094).

15 The expression cassette according to the invention can therefore comprise, for example, a seed-specific promoter (preferably the phaseolin promoter, the USP or LEB4 promoter), the LEB4 signal peptide, the gene to be expressed and an ER retention signal.

20 The inserted nucleotide sequence coding for a GMP synthetase can be prepared synthetically or be obtained naturally or comprise a mixture of synthetic and natural DNA components. In general, synthetic nucleotide sequences are produced with codons preferred by plants. These codons preferred by plants can be identified by

25 codons which have the highest protein frequency and are expressed in most plant species of interest. To prepare an expression cassette it is possible to manipulate various DNA fragments in order to obtain a nucleotide sequence which expediently reads in the correct direction and is equipped with a correct reading

30 frame. Adaptors or linkers can be attached to the framework to connect the DNA fragments together.

Artificial DNA sequences are also suitable as long as they confer, as described above, the required property of increasing

35 the content of guanosine nucleotides in the plant through overexpression of the GMP synthetase gene in crop plants. Such artificial DNA sequences can be found, for example, by translation back from proteins having GMP synthetase activity and constructed by molecular modeling, or by *in vitro* selection.

40 Particularly suitable coding DNA sequences are those obtained by translation back from a polypeptide sequence in accordance with the codon usage specific for the host plant. The specific codon usage can easily be found by a skilled worker familiar with methods of plant genetics by computer analyses of other known

45 genes of the plant to be transformed.

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Further suitable equivalent nucleic acid sequences according to the invention which may be mentioned are sequences coding for fusion proteins where one constituent of the fusion protein is a plant GMP synthetase polypeptide or a functionally equivalent portion thereof. The second part of the fusion protein can be, for example, another polypeptide with enzymatic activity or an antigenic polypeptide sequence with whose aid it is possible to detect GMP synthetase expression (e.g. myc tag or his tag). However, this is preferably a regulatory protein sequence such as, for example, a signal or transit peptide which guides the GMP synthetase protein to the desired site of action.

The promoter regions according to the invention and the terminator regions ought expediently to be provided in the direction of transcription of a linker or polylinker containing one or more restriction sites for insertion of this sequence. As a rule, the linker has 1 to 10, usually 1 to 8, preferably 2 to 6, restriction sites. The size of the linker within the regulatory region is generally less than 100 bp, frequently less than 60 bp, but at least 5 bp. The promoter according to the invention may be both native or homologous and foreign or heterologous to the host plant. The expression cassette according to the invention comprises in the 5'-3' direction of transcription the promoter according to the invention, any suitable sequence and a region for the transcriptional termination. Different termination regions can be exchanged for one another if desired.

It is furthermore possible to employ manipulations which provide suitable restriction cleavage sites or delete the excess DNA or restriction cleavage sites. Where the insertions, deletions or substitutions such as, for example, transitions and transversions are considered, it is possible to use *in vitro* mutagenesis, primer repair, restriction or ligation. In the case of suitable manipulations such as, for example, restriction, chewing-back or filling in of overhangs for blunt ends, it is possible to make complementary ends of the fragments available for the ligation.

Preferred polyadenylation signals are plant polyadenylation signals, preferably those which essentially correspond to T-DNA polyadenylation signals from *Agrobacterium tumefaciens*, in particular of gene 3 of the T-DNA (octopine synthase) of Ti plasmid pTiACH5 (Gielen et al., EMBO J. 3 (1984) 835 ff) or functional equivalents.

To transform a host plant with a DNA coding for a GMP synthetase, an expression cassette according to the invention is incorporated

as insert into a recombinant vector whose vector DNA contains additional functional regulatory signals, for example sequences for replication or integration. Suitable vectors are described inter alia in "Methods in Plant Molecular Biology and

5 Biotechnology" (CRC Press), Chapters 6/7, pages 71-119.

The transfer of foreign genes into the genome of a plant is referred to as transformation. The methods used for this purpose are those described for the transformation and regeneration of

10 plants from plant tissues or plant cells for transient or stable transformation. Suitable methods are protoplast transformation by polyethylene glycol-induced DNA uptake, the biolistic approach with the gene gun, electroporation, incubation of dry embryos in DNA-containing solution, microinjection and gene transfer

15 mediated by agrobacterium. The methods mentioned are described, for example, in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press (1993) 128-143 and in Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42

20 (1991) 205-225). The construct to be expressed is preferably cloned into a vector which is suitable for transforming *Agrobacterium tumefaciens*, for example pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984) 8711).

25 *Agrobacteria* transformed with an expression cassette according to the invention can likewise be used in known manner for transforming plants, especially plants such as cereals, corn, soybean, rice, cotton, sugarbeet, canola, sunflower, flax, hemp, potato, tobacco, tomato, oilseed rape, alfalfa, lettuce and

30 various tree, nut and vine species, and legumes, for example by bathing wounded leaves or pieces of leaf in a solution of *agrobacteria* and then cultivating in suitable media.

The site of pyrimidine biosynthesis is generally the leaf tissue,

35 so that leaf-specific expression of the GMP synthetase gene is sensible. However, it is obvious that pyrimidine biosynthesis need not be confined to leaf tissue but may also take place in all other parts of the plant, for example in fat-containing seeds, tissue-specifically.

40

In addition, constitutive expression of the exogenous GMP synthetase gene is advantageous. However, on the other hand, inducible expression may also appear desirable.

45 Using the recombinant and cloning techniques quoted above, the expression cassettes according to the invention can be cloned into suitable vectors which make it possible to replicate them,

for example into E. coli. Suitable cloning vectors are, inter alia, pBR322, pUC series, M13mp series and pACYC184. Binary vectors able to replicate both in E. coli and in agrobacteria are particularly suitable.

5

A further aspect of the invention relates to the use of an expression cassette according to the invention for transforming plants, plant cells, plant tissues or parts of plants. The aim of the use is preferably to increase the GMP synthetase content in

10 the plant.

This may involve, depending on the chosen promoter, expression specifically in the leaves, in the seeds or other parts of the plant. Such transgenic plants, their propagation material and

15 their plant cells, tissues or parts form a further aspect of the present invention.

The invention is illustrated by the Examples which now follow, but is not confined to these:

20

#### Examples

Methods of genetic manipulation on which the examples are based:

25 

#### General cloning methods

Cloning methods such as, for example, restriction cleavage, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes,

30 linkage of DNA fragments, transformation of Escherichia coli cells, cultivation of bacteria and sequence analysis of recombinant DNA were carried out as described by Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6).

35 

#### Sequence analysis of recombinant DNA

Recombinant DNA molecules were sequenced using a laser fluorescence DNA sequencer supplied by ABI by the method of Sanger (Sanger et al. (1977), Proc. Natl. Acad. Sci. USA 74,

40 5463-5467). Fragments resulting from a polymerase chain reaction were sequenced and checked to avoid polymerase errors in constructs to be expressed.

The chemicals used were purchased, unless otherwise mentioned, in

45 analytical quality from Fluka (Neu-Ulm), Merck (Darmstadt), Roth (Karlsruhe), Serva (Heidelberg) and Sigma (Deisenhofen). Solutions were made up using prepared, pyrogen-free water,

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referred to as H<sub>2</sub>O in the subsequent text, from a Milli-Q water treatment system (Millipore, Eschborn). Restriction endonucleases, DNA-modifying enzymes and molecular biology kits were purchased from AGS (Heidelberg), Amersham (Braunschweig),  
 5 Biometra (Göttingen), Roche (Mannheim), Genomed (Bad Oeynhausen), New England Biolabs (Schwalbach/Taunus), Novagen (Madison, Wisconsin, USA), Perkin-Elmer (Weiterstadt), Pharmacia (Freiburg), Qiagen (Hilden) and Stratagene (Heidelberg). They were used in accordance with the manufacturers' instruction  
 10 unless mentioned otherwise.

The strains of bacteria used hereinafter (*E. coli*, XL-1 Blue) were purchased from Stratagene. *E. coli* AT 2465 was purchased from the coli genetic stock center (Yale University, New Haven).  
 15 The agrobacterial strain used for the plant transformation (*Agrobacterium tumefaciens*, C58C1 with the plasmid pGV2260 or pGV3850kan) has been described by Deblaere et al. (Nucl. Acids Res. 13 (1985) 4777). An alternative possibility is also to employ the agrobacterial strain LBA4404 (Clontech) or other  
 20 suitable strains. Vectors which can be used for the cloning are pUC19 (Yanish-Perron, Gene 33(1985), 103-119) pBluescript SK- (Stratagene), pGEM-T (Promega), pZero (Invitrogen), pBin19 (Bevan et al., Nucl. Acids Res. 12(1984) 8711-8720) and pBinAR (Höfgen and Willmitzer, Plant Science 66 (1990) 221-230).

## 25 Example 1

Isolation of a cDNA of the *guaA* gene, coding for a GMP synthetase from tobacco.

30 An expressed sequence tag (EST) from *Arabidopsis thaliana* (EST F14426) which, on a partial reading frame, encodes a polypeptide of 68 amino acids with 60% similarity with a GMP synthetase from *Helicobacter pylori* was subjected to partial 5'-terminal  
 35 sequencing. The oligonucleotides 5'-aag gat cca agc tct aag acc cta tcc-3' and 5'-tta gat ctt tat tcc cat tcg atg g-3' from the 5'- and 3'-terminal sequences were used for amplification by a polymerase chain reaction (PCR) of a 1000 bp cDNA fragment with EST F14426 as template in a Perkin Elmer DNA thermal cycler. The  
 40 reaction mixture contained 0.1 ng/μl cDNA from tobacco, 0.5 μM of the appropriate oligonucleotides, 200 μM nucleotides (Pharmacia), 50 mM KCl, 10 mM Tris-HCl (pH 8.3 at 25°C, 1.5 mM MgCl<sub>2</sub>) and 0.02 U/μl Taq polymerase (Perkin Elmer).

45 The amplification conditions were set as follows:

Annealing temperature: 52°C, 1 min

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Denaturation temperature: 92°C, 1 min  
Elongation temperature: 72°C, 1.5 min  
Number of cycles: 30

- 5 The fragment was employed for screening a cDNA library from callus tissue of *Nicotiana tabacum* (variety Samsun NN) in the vector ZAP Express. For this purpose,  $2.5 \times 10^5$  lambda phages from the cDNA library were plated out on agar plates with *E. coli* XL1-Blue as bacterial strain. The phage DNA was transferred by  
10 standard methods (Sambrook et al. (1989); Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6) to nitrocellulose filters (Gelman Sciences) and fixed on the filters. The hybridization probe used was the PCR fragment described above which had been radiolabeled using a multiprime DNA labeling system (Amersham  
15 Buchler) in the presence of  $\alpha$ - $^{32}\text{P}$ -dCTP (specific activity 3000 Ci/mmol) in accordance with the manufacturer's information. The hybridization of the membranes took place after prehybridization at 60°C in 3 x SSPE, 0.1% sodium dodecyl sulfate (w/v), 0.02% polyvinylpyrrolidone (w/v), 0.02% Ficoll 400 (w/v)  
20 and 50 mg/ml calf thymus DNA for 12-16 hours (Sambrook et al. (1989); Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6). The filters were then washed in 2 x SSPE, 0.1% sodium dodecyl sulfate (w/v) at 60°C for 60 minutes. Positively hybridizing phages were visualized by autoradiography and purified and  
25 isolated by standard techniques.

- It was possible to identify and purify 13 hybridizing signals. After restriction analysis, the clones GMP-6 and GMP-7M were selected for double-stranded sequencing. Evaluation of the  
30 sequencing data showed that the clone GMP-7M with a length of 1973 bp contained a complete reading frame of 1614 bp which codes for a protein of 538 amino acids with a calculated molecular weight of 60.1 kDa (SEQ-ID No. 1). In front of the presumed start codon there is a stop codon in the same reading frame, which  
35 suggests that GMP-7M is a full-length cDNA. GMP-7M thus represents the first full-length plant cDNA of a GMP synthetase. GMP-6 is a partial clone which is 217 nucleotides shorter than GMP-7M on the 5'. GMP-7M shows similarities with GMP synthetases from microorganisms and animals. Apart from the partial amino  
40 acid sequence encoded on EST F14426 there are no other sequences from plants with homology with GMP synthetases in the databases. The greatest similarity (62%) is with a GMP synthetase from *Helicobacter pylori*. It is also evident that the similarities between the C termini of the GMP synthetases are greater than  
45 those in the region of the N termini. The N terminus of the GMP-7M amino acid sequence corresponds with the N termini of GMP synthetases from other organisms such as *E. coli* and

*Synechocystis* sp. (Table 1). GMP-7M has no marked signal sequences (found by the program PSORT, Nakai, K., Institute for Molecular and Cellular Biology, Osaka University, Japan), which might indicate a cytosolic localization of the protein.

5

Table 1

Sequence comparison of GMP synthetases from *Nicotiana tabacum* (guaA\_N.t = GMP-7M), *Arabidopsis thaliana* (guaA\_est\_A.t, Genbank No. F14426), *E.coli* (guaA\_e.c, Genbank No. 146276), *Synechocystis* sp. (guaA\_syn, Genbank No. 1001583), *Helicobacter pylori* (guaA\_h.p, Genbank No. 3122166), *Homo sapiens* (guaA\_human, Genbank No. 1708072).

15		1			50
	guaA_N.t	-----	-----MEPQ	TQAKKSNLVL	ILDYGSQYTH LITRRIRSL
	guaA_est_A.t	-----	-----	-----	-----
	guaA_e.c	-----	-----m	tenihkhril	ildfgsqytg lvarrvrelg
	guaA_syn	mttqipvppv	vsdqalpdri	sdrkkgqiiv	ildfgsqyse liarrirete
	guaA_h.p	-----	-----	-----mil	vldfgsqytg liarrlrerg
20	guaA_human	-malcngdsk	lenaggdlkd	ghhhyegavv	ildagaqygk vidrrvrelf
		51			100
	guaA_N.t	IFSLTINGTS	SLDSIKELDP	RVIILSGGPH	SVHADGAPCF PPGFIEYVES
	guaA_est_A.t	-----	-----	-----	-----
	guaA_e.c	vycelwawdv	teaqirdfnp	sguilsggpe	stteenspra pq....yvfe
	guaA_syn	vysevlsyrt	taqqlreikp	kgiilsggpn	svydgapec dp....eifq
	guaA_h.p	iyteivpffe	sieniqkkap	kglilsggpa	svyakdaykp sg....kifd
25	guaA_human	vqseifplet	pafaikeggf	raiiisggpn	svyaedapwf dpa....ift
		101			150
	guaA_N.t	RGIHVLGICY	GLQLIVQKLG	GVVKIGEKHE	YGRMEIEVGK NVV....GGL
	guaA_est_A.t	-----	-----	-----	-----
	guaA_e.c	agvpvfgvcy	gmqtmmamlg	ghveasnere	fgyaqvevvn dsalvrgied
	guaA_syn	lgvpvlgvcy	gmqlmvkqlg	grverakrge	ygkaslhidd ptdlltnven
30	guaA_h.p	lnvpilgicy	gmqylvdffg	gvvvganeqe	fgkavleitq nsvifegv..
	guaA_human	igkpvlgicy	gmqmmnkfvf	gtvhkksvre	dgvfnisvsn tcslfrglqk
		151			200
	guaA_N.t	FGNTEIGDKQ	VVWMSHGDEA	VKLPEGFVV	ARSSQGAVAA IENRERRFYG
	guaA_est_A.t	-----	-----	-----	-----
	guaA_e.c	altadgkpll	dvwmshgdkv	taipsdfitv	astescpfai maneekrfyg
35	guaA_syn	dst.....	.mwmsbgdsc	vdltptgfeil	ahtdntpcaa iadhqkalfg
	guaA_h.p	.....kiks	lvwmshmdkv	ielpkgfttl	akspnsphca iengk..ifg
	guaA_human	.....ee	vllthgds	dkvadgfkvv	arsgni.vag ianeskklyg
		201			250
	guaA_N.t	LQYHPEVTHS	TEGMRTLRFH	LFDVCGVTAG	WKMEDVLEEE IKVIKGMVGP
	guaA_est_A.t	-----	-----	-----	-----
	guaA_e.c	vqfhpevtht	rqqmrmlerf	vrldicqceal	wtpakiidda varireqvg.
40	guaA_syn	vqfhpevvhs	vggialirnf	vyhichcept	wttaafiees irevrsqvg.
	guaA_h.p	lqfhpevvqs	eeggkilenf	allvcgcekt	wgmghfaqre iarlekia.
	guaA_human	aqfhpevglt	engkvilknf	lydiagcsst	ftvqnrelec ireikervgt
		251			300
	guaA_N.t	EDHVICALSG	GVDSTVAAKL	VHKAIG.DRL	HCVFVDNGLL RYKERERVME
	guaA_est_A.t	-----	-----	-----	-----
45	guaA_e.c	ddkvilglsg	gvdssvtaml	lhraig.knl	tcvfvdnll rlnaeqvld
	guaA_syn	drvllalsg	gvdssstlafl	lhraig.dnl	tcmfidqgfm rkgeperlve
	guaA_h.p	nakvlcavsg	gvdstvvtal	lhraik.dnl	iavfvdhgll rknekervqa
	guaA_human	s.kvlvllsg	gvdstvctal	lnralnqeqv	iavhidngfm rkresqsvee

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		301		350
	guaA_N.t	LF EK.....	...RLHLPVT	CVDATEEFLS KLKGVTEPEM
	guaA_est_A.t	-----	-----	-----
	guaA_e.c	mfgd.....	...hfglniv	hvpadrfls alagendpea
	guaA_syn	lf dh.....	...qfhipvq	yvnardrflk qlegvtdpee
5	guaA_h.p	mfdk.....	...lkipln	tidakevfls klkgvsepel
	guaA_human	alkklgiqvk	vinaahsfyn	gtttlpisde drtprkrisk tlnmttspee
		351		400
	guaA_N.t	KRKIIIGKEFI	NIFDLFAHDV	EEKVGKKPSY LVQGTLYPDV IESC...PPP
	guaA_est_A.t	-----	-----	-----
	guaA_e.c	krkiigrvfv	evfd..eeal	k...ledvkw laqgtiypdv iesaas....
10	guaA_syn	krriighefi	qvfe..eesn	r...lgpfdy laqgtlypdv iesadsnvpd
	guaA_h.p	krkiigetfi	evfe..keak	khhlkgkief laqgtlypdv iesvsv....
	guaA_human	krkiigdtfv	ki..anevig	emnlkpeevf laqgtlrrpd iesa sl....
		401		450
	guaA_N.t	GSGRTHSHTI	KSHHNVGGLP	KDMKL..KLI EPLKLLFKDE VREL GKILDI
	guaA_est_A.t	-----	-----	-----
	guaA_e.c	atgk..ahvi	kshhnvggpl	kemkm..glv eplkelfkde vrkiglelgl
15	guaA_syn	ktgervavki	kshhnvggpl	knlr f..klv eplrklfkde vrklgrsigl
	guaA_h.p	...kgpskvi	kthhnvggpl	ewmdf..kli eplrelfkde vrllgkelgv
	guaA_human	.vasgkaeli	kthhndteli	rklreegkvi eplkdfhkde vrilgrelgl
		451		500
	guaA_N.t	SEDFLKRHPF	PGPGLAVRIP	GDVTAGNSLD ILRQVDEIFI QSIRDAKIYD
	guaA_est_A.t	-----	-----	-----
20	guaA_e.c	pydmlyrhpf	pgpglgvrvi	gevk k.eycd llrradaifi eelrkadlyd
	guaA_syn	peeivrrhpf	pgpglairii	gevts.erln ilrdadfivr deiskrgiyh
	guaA_h.p	sqdflmrhpf	pgpglavril	geise.skik rlqeadfifi eelkkanlyd
	guaA_human	peelvsrhpf	pgpglairvi	c.aeepyick dfpetnnilk ivadfsasvk
		501		550
	guaA_N.t	EIWQAFVFL	PVKTVGVQGD	QRTSHAVAL RA.VTSQDGM TADWYYFDFK
25	guaA_est_A.t	-----	---aggd	kgtiphvgcp pcr lqaqvg l tadwfifehk
	guaA_e.c	kvsqaftvfl	prsvvgvmgd	grkydwvvs l ra.vetidfm tahwahlpyd
	guaA_syn	dywqafavll	pirsvgvmgd	krtyahpvvl rf.itsedgm tadwarvpd
	guaA_h.p	kwvqafcvll	nvsvvgvmgd	nrtynaicl ra.vnasdgm tasfsflehs
	guaA_human	kphtllqrvk	actteedqek	lmqitslhsl nafllpiktv gvqgdcrsys
		551		600
	guaA_N.t	FLDDVSRKIC	NSVRGVNRVL	LDITSKPPST IEWE-----
30	guaA_est_A.t	flddvsrkic	nsvqgvnrvi	lditskppst iewe-----
	guaA_e.c	flgrvsnrri	nevngisrvv	ydisgkppat iewe-----
	guaA_syn	ilea isnriv	nevgvnrvi	yditskppgt iewe-----
	guaA_h.p	flekvsnrri	nevsginrvv	yditskppgt iewe-----
	guaA_human	yvcgisskde	pdweslfla	rliprmchnv nrsvyifgpp vkep ptdvtp
		601		650
35	guaA_N.t	-----	-----	-----
	guaA_est_A.t	-----	-----	-----
	guaA_e.c	-----	-----	-----
	guaA_syn	-----	-----	-----
	guaA_h.p	-----	-----	-----
	guaA_human	tflttgvlst	lrqadfeahn	ilresgyagk isqmpviltp lhfd rdp lqk
		651		700
40	guaA_N.t	-----	-----	-----
	guaA_est_A.t	-----	-----	-----
	guaA_e.c	-----	-----	-----
	guaA_syn	-----	-----	-----
	guaA_h.p	-----	-----	-----
	guaA_human	qp scqrsvvi	rtf it sdfmt	gipatpgnei pvevvlkmvt eikk ipgisr
45		701	716	
	guaA_N.t	-----	-----	-----
	guaA_est_A.t	-----	-----	-----
	guaA_e.c	-----	-----	-----

guaA\_syn -----  
 guaA\_h.p -----  
 guaA\_human imydltskpp gttewe  
 Example 2

- 5 Isolation of a cDNA of the guaA gene, coding for a GMP synthetase from the moss *Physcomitrella patens*

Double-stranded cDNA was generated from mRNA from protonemata of various ages of *Physcomitrella patens* and used to produce a cDNA bank in the vector pBluescript SKII (lambda ZAP II RI Library construction kit, Stratagene). Single clones from this bank were partially sequenced. The sequence of the clone 093-d11 showed clear homology with the GMP synthetase from *Aquifex aeolicus*. The complete sequence of 093-d11 was determined, see SEQ-ID No. 3.

- 10  
 15 093\_d11 has a length of 1232 nucleotides and codes on a continuous reading frame for 382 amino acids. Comparison with GMP-7M reveals that 093\_d11 is a partial cDNA. The homology with GMP-7M is 66.7% at the nucleotide level and 74.6% at the amino acid level.

- 20  
 Example 3

Demonstration of the function of GMP-7M by complementation of *E. coli*

- 25  
 The GMP-7M cDNA was employed as template for a PCR with the oligonucleotides 5'-CCTAGCCATGGAACCTCAAAC-3' and 5'-TATAGGATCCTACTTTGGTCACC-3'. The reaction mixtures contained about 0.1 ng of GMP-7M DNA, 0.5 µM of the appropriate  
 30 oligonucleotides, 200 µM nucleotides (Pharmacia), 50 mM KCl, 10 mM Tris-HCl (pH 8.3 at 25°C, 1.5 mM MgCl<sub>2</sub>) and 0.02 U/µl Pfu polymerase (Stratagene).

- 35 The amplification conditions were set as follow:

- Annealing temperature: 50°C, 30 sec  
 Denaturation temperature: 92°C, 30 sec  
 Elongation temperature: 72°C, 3 min  
 40 Number of cycles: 25

- The resulting fragment of about 1670 bp was ligated via the NcoI and BamHI cleavage sites introduced by the oligonucleotides into the vector pTrc99A (Pharmacia). The resulting construct GMP-7Trc was transformed into the *E. coli* strain AT2465 (genetic markers:  
 45 thi-1, guaA21, relA1, λ, spoT1) and plated out on M9 minimal medium (Sambrook et al. (1989) Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6) with and without 100 µg/ml guanosine.



## 20

- The minimal media contained 0.4% glucose, 0.2% casamino acids, 100 µg/ml thiamine, 100 µg/ml inosine, 100 µg/ml biotin, 100 µg/ml histidine, 100 µg/ml arginine, 100 µg/ml 2'-deoxyuridine, 100 µM IPTG and 25 µg/ml ampicillin. The cloning vectore pTrc99A was
- 5 transformed into AT2465 in a parallel experiment. It emerged that only the transformed bacteria which contained a GMP-7M cDNA from tobacco in the expression vector pTrc99A were capable of growth on minimal media without guanosine (see Tab. 2), which points strongly to the GMP-7M cDNA coding for an active GMP synthetase.
- 10 The enzyme encoded by GMP-7M thus represents the first functional GMP synthetase isolated from plants.

Table 2

- 15 Growth of E. coli AT2465 transformed with various plasmids after 2 days at 37°C

	pTrc99A + GMP-7M	pTrc99A
20 Minimal medium without guanosine	+	-
Minimal medium with guanosine (100 µg/ml)	+	+

## 25 Example 4

Overexpression of the GMP synthetase from tobacco in E.coli and production of antibodies

- 30 For overexpression in E.coli, BamHI cleavage sites were introduced by PCR with GMP-7M as template and the oligonucleotides GMPA: 5'-GCAATGGATCCTCAAACACAGGCG-3' and GMPB: 5'-AAAAGGATCCTACTTTGGTCACC-3' and made it possible to clone the fragment in the vector pET15b (Novagen). A GMP-7M reading frame
- 35 with hexahistidine anchor at the N terminus was produced in this way. After the correct orientation had been checked by restriction digestion and polymerase errors had been excluded by sequencing, the resulting construct GMP-7E was transformed into E. coli BL21(DE3) (Stratagene). IPTG-induced one-day cultures
- 40 were harvested by centrifugation, and the cell pellets were lyzed and treated further in accordance with the manufacturer's information for nickel affinity chromatography ("Qia-Express-Kit", Qiagen). It was possible in this way to purify the GMP synthetase to more than 95% purity. The protein
- 45 was used for producing antisera in rabbits by conventional

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protocols (carried out on contract by Eurogentec, Herstal, Belgium).

## Example 5

5

Expression of the GMP synthetase from tobacco in baculovirus-infected insect cells

In order to obtain sufficient active GMP synthetase for mass testing of chemicals, a 1.65 kb fragment was excized from GMP-7E with BamHI and cloned into the transfer vector pFastBacHTa (GibcoBRL). The resulting construct GMP-7I was used to generate recombinant baculovirus in accordance with the manufacturer's information (GibcoBRL). This virus was used in accordance with the manufacturer's information (GibcoBRL) for infecting Sf21 insect cells in order to produce active GMP synthetase whose activity could be measured after disruption of the cells in 50 mM Tris-HCl, pH 7.6, 10 mM KCl, 1 mM EDTA, 10 mM PMSF and desalting of the extract on a Sephadex G-25 column (Pharmacia, Sweden).

20

## Example 6

Production of plant expression cassettes

The antisense and cosuppression techniques were used with the aim of reducing the GMP synthetase activity in transgenic tobacco plants. For this purpose, plasmid constructs were produced in the vector pBinAR (Höfgen and Willmitzer, Plant Science (1990) 66, 221-230). A fragment of 1599 bp obtained from GMP-7M with BamHI and BglII was ligated into the BamHI-cut vector pBinAR. The 1599 bp fragment encodes the 5'-terminal part of the GMP synthetase cDNA. Clones obtained after transformation into E.coli XL1-blue were examined for the orientation of the 1599 cassette by cutting with HindIII as a check. The plasmids pGMP7AS (antisense construct) and pGMP7EX (sense construct) were identified in this way, see Figure 2.

## Example 7

40 Generation and analysis of transgenic plants

The plasmids pGMP7AS and pGMP7EX - see Figure 2 - were transformed into Agrobacterium tumefaciens C58C1:pGV2260 (Deblaere et al., Nucl. Acids. Res. 13(1984), 4777-4788). A 1:50 dilution of an overnight culture of a positively transformed agrobacterial colony in Murashige-Skoog medium (Physiol. Plant. 15(1962), 473) with 2% sucrose (2MS medium) was used for

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- transforming tobacco plants (*Nicotiana tabacum* cv. Samsun NN). Leaf disks from sterile plants (each about 1 cm<sup>2</sup>) were incubated in a Petri dish with a 1:50 agrobacteria dilution for 5-10 minutes. This was followed by incubation on 2MS medium with 0.8% Bacto agar in the dark at 25°C for 2 days. Cultivation was continued after 2 days with 16 hours light/8 hours dark, and continued in a weekly rhythm on MS medium with 500 mg/l Claforan (cefotaxime sodium), 50 mg/l kanamycin, 1 mg/l benzylaminopurine (BAP), 0.2 mg/l naphthylacetic acid and 1.6 g/l glucose. Growing shoots were transferred to MS medium with 2% sucrose, 250 mg/l Claforan and 0.8% Bacto agar. Regenerated shoots were obtained on 2MS medium with kanamycin and Claforan and, after rooting, were transferred into soil and, after cultivation for two weeks in a controlled-environment cabinet with a 16 hour light/8 hour dark rhythm at 60% humidity, investigated for foreign gene expression and altered metabolite contents and phenotypical growth traits. Altered nucleotide contents can be determined, for example, by the method of Stitt et al. (FEBS Letters, 145 (1982), 217-222).
- 20 The transgenic GMP synthetase antisense plants and their filial generation showed reduced growth, compared with WT plant controls, and bleaching of the sink leaves. These phenotypical changes occurred in an early growth stage (see Fig. 3). In plants with reduced growth it was possible to detect in Northern
- 25 hybridization a reduced amount of GMP-7M RNA, compared with the wild type. 40 µg portions of complete RNA from sink leaves were employed for this purpose. Complete RNA was isolated from plant tissues as described by Logemann et al. (Anal. Biochem. 163 (1987), 21). For the analysis in each case 40 µg of RNA were
- 30 fractionated in a formaldehyde-containing 1.5% agarose gel and transferred to nylon membranes (Hybond, Amersham). Specific transcripts were detected as described by Amasino (Anal. Biochem. 152(1986), 304). A specific c-DNA probe of the antisense strand was generated. This was done by cleaving the plasmid GMP-7M with
- 35 BamHI and BglII and isolating a fragment comprising 1600 bp. The oligonucleotide 5'-GAT ACG TCG TCA AGG AAC TTG-3' was used for the labeling reaction. The probe was hybridized by standard methods, see Hybond information for users, Amersham. Hybridization [sic] signals were visualized by autoradiography
- 40 using Kodak X-OMAT AR films. A clear correlation between expression of the growth phenotype and a reduction in the amount of GMP-7M RNA was shown (Fig. 3).

It was moreover possible in a Western blot experiment to detect a

45 reduced amount, compared with wild-type plants, of GMP synthetase in the transgenic lines. This was done by preparing total protein extracts from sink leaves, separating in SDS polyacrylamide gel

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electrophoresis by standard methods and transferring to nitrocellulose membranes. Detection took place with an IgG-alkaline phosphatase conjugate and the BCIP/NBT system (Sigma).

5

In addition, it was possible by the in vitro assay described in Example 8 to establish that there was reduced GMP synthetase activity in transgenic lines with reduced growth.

- 10 The correlation between the level of expression and the GMP synthetase activity and growth phenotype suggest that GMP synthetase is a suitable target for herbicides.

## Example 8

15

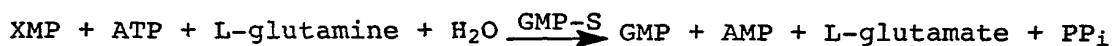
## Assay systems for measuring GMP synthetase activity

The systems developed by Spector (Methods in Enzymology LI, 1978, 219-224) for animal enzymes can be used to measure plant GMP

- 20 synthetase activity. In the first system, the AMP formation is made possible by coupling the reaction with AMP kinase, pyruvate kinase, lactate dehydrogenase and measurement at 340 nm. The second system is based on direct detection of GMP (guanosine monophosphate) by employing the radiolabeled substrate XMP  
25 (xanthine monophosphate) and fractionation by thin-layer chromatography.

Alternatively, the GMP synthetase activity can also be measured by a novel system, namely coupled detection of the produced

- 30 glutamate. This system has the advantage of a smaller number of coupled reaction steps and provides greater signal strengths.



35



- (GMP-S = GMP synthetase, GluDH = glutamate dehydrogenase, APAD =  
40 3-acetylpyridine adenine dinucleotide)

- For this, the reaction mixture (see below) was incubated at 37°C for 60 minutes, and the reaction was stopped by incubation at 95°C for 5 minutes. The glutamate formed was detected in the detection  
45 mixture (see below) by photometric measurement of the increase in APADH at 363 nm.

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## Reaction mixture:

	100 $\mu$ L	750 mM	Tris/HCl buffer pH 7.8
	100 $\mu$ L	100 mM	MgCl <sub>2</sub>
5	100 $\mu$ L	80 mM	KCl
	100 $\mu$ L	20 mM	XMP
	100 $\mu$ L	200 mM	L-glutamine
	400 $\mu$ L		H <sub>2</sub> O
	<u>100 <math>\mu</math>L</u>		protein extract
10	1000 $\mu$ L		

## Detection mixture:

	375 $\mu$ L	100 mM	Tris-HCl buffer pH 8.0
15	75 $\mu$ L	500 mM	KCl
	125 $\mu$ L		H <sub>2</sub> O
	75 $\mu$ L	3 mM	APAD
	<u>100 <math>\mu</math>L</u>		of the reaction mixture
	750 $\mu$ L		

20

## Example 9

## Search for inhibitors of GMP synthetase activity

- 25 The *in vitro* assay described in Example 8 can be used with high throughput methods to search for inhibitors of GMP synthetase activity. The GMP synthetase activity for this can be prepared from plant tissues. It is possible and preferred for a plant GMP synthetase to be expressed in *E. coli*, insect cells or another suitable expression system and then be concentrated or isolated. It was possible in this way to identify known inhibitors such as 6-thio-XMP.

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We claim:

- 5 1. A DNA sequence comprising the coding region of a plant GMP synthetase, wherein this DNA sequence has the nucleotide sequence SEQ-ID No: 1 or SEQ-ID No: 3.
- 10 2. A DNA sequence which hybridizes with the DNA sequence SEQ-ID No: 1 or SEQ-ID No: 3 as claimed in claim 1 or parts thereof or derivatives derived from these sequences by insertion, deletion or substitution, and codes for a protein which has the biological activity of a GMP synthetase, this DNA sequence having a homology of at least 60% with SEQ ID NO: 1.
- 15 3. A protein having GMP synthetase activity and comprising an amino acid sequence which represents a portion of at least 100 amino acids of the sequence SEQ-ID No: 2 or 4.
- 20 4. A protein as claimed in claim 3, which comprises as amino acid sequence the part-sequence 50 - 300 from SEQ-ID No: 2 or SEQ-ID No: 4.
- 25 5. A protein as claimed in claim 4, which comprises as amino acid sequence the sequence depicted in SEQ-ID No: 2 or SEQ-ID No: 4.
- 30 6. The use of a DNA sequence as claimed in claim 1 or 2 for introduction into pro- or eukaryotic cells, this sequence optionally being linked to control elements which ensure transcription and translation in the cells, and leading to expression of a translatable mRNA which brings about the synthesis of a plant GMP synthetase.
- 35 7. The use of a DNA sequence as claimed in claim 1 or 2 for producing an assay system for identifying inhibitors of plant GMP synthetase with a herbicidal action.
- 40 8. A method for finding substances which inhibit the activity of plant GMP synthetase, which comprises in a first step using a DNA sequence as claimed in claim 1 or 2 preparing GMP synthetase, and in a second step measuring the activity of the plant GMP synthetase in the presence of a test substance.
- 45

9. A method as claimed in claim 8, wherein the measurement of the plant GMP synthetase is carried out in a high throughput screening (HTS).

5 10. A method for identifying substances with a herbicidal action, which inhibit the GMP synthetase activity in plants, consisting of

10 a) preparation of transgenic plants, plant tissues, or plant cells which comprise an additional DNA sequence coding for an enzyme having GMP synthetase activity and are able to overexpress an enzymatically active GMP synthetase;

15 b) application of a substance to transgenic plants, plant cells, plant tissues or plant parts and to untransformed plants, plant cells, plant tissues or plant parts;

20 c) determination of the growth or survivability of the transgenic and untransformed plants, plant cells, plant tissues or plant parts after application of the chemical substance; and

25 d) comparison of the growth or survivability of the transgenic and untransformed plants, plant cells, plant tissues or plant parts after application of the chemical substance;

30 where suppression of the growth or survivability of the untransformed plants, plant cells, plant tissues or plant parts without, however, greatly suppressing the growth or the survivability of the transgenic plants, plant cells, plant tissues or plant parts demonstrates that the substance from b) shows herbicidal activity and inhibits the enzymic activity in plants.

35

40 11. An assay system based on the expression of a DNA sequence SEQ-ID No. 1 or SEQ-ID No. 3 as claimed in claim 1 or 2 for identifying inhibitors of plant GMP synthetase with a herbicidal action.

45 12. An assay system as claimed in claim 11 for identifying inhibitors of plant GMP synthetase, wherein the enzyme is incubated with a test substrate to be investigated and, after a suitable reaction time, the enzymatic activity of the enzyme is measured by comparison with the activity of the

uninhibited enzyme.

13. An inhibitor of plant GMP synthetase.

5 14. An inhibitor of plant GMP synthetase identified using an assay system as claimed in claim 11 or 12.

10 15. An inhibitor as claimed in either of claims 13 or 14 for use as herbicide.

15 16. A method for eliminating unwanted plant growth, which comprises treating the plants to be eliminated with a compound which specifically binds to GMP synthetase encoded by a DNA sequence as claimed in claim 1 or 2, and inhibits the function thereof.

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GMP synthetase from plants

5 Abstract

The present invention relates to a DNA coding for a polypeptide having GMP synthetase (EC 6.3.5.2) activity. The invention additionally relates to the use of this nucleic acid for  
10 producing an assay system.

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FIG. 1

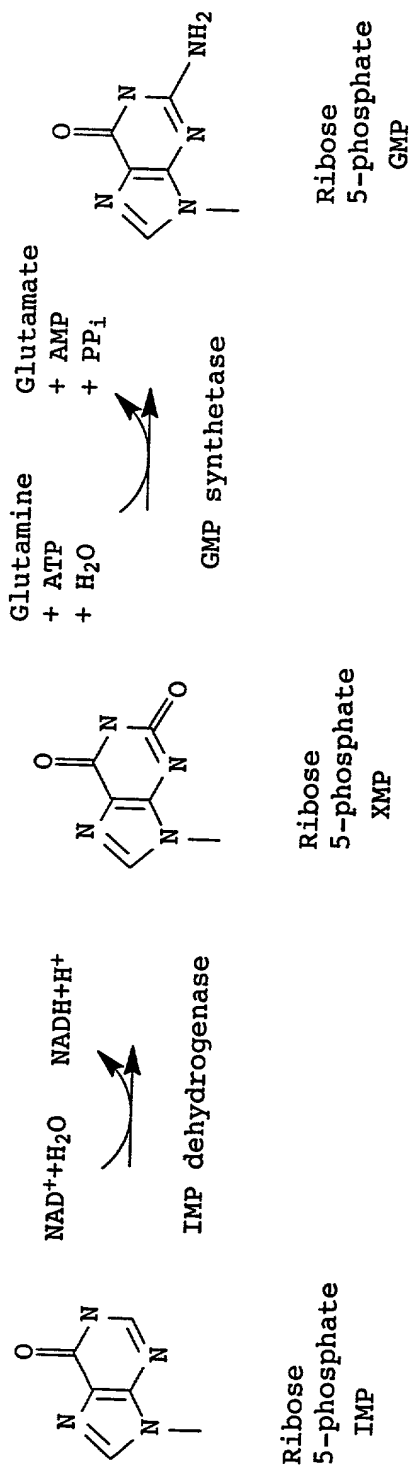


FIG. 2

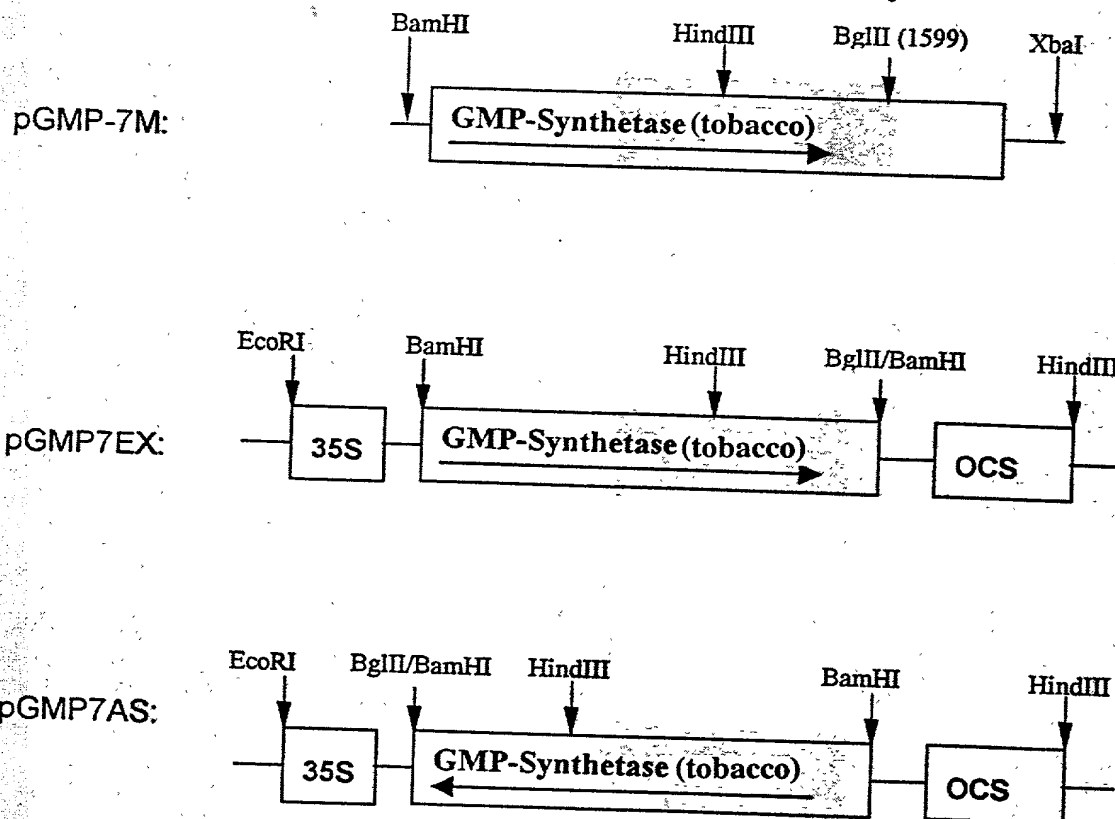


Fig. 3:

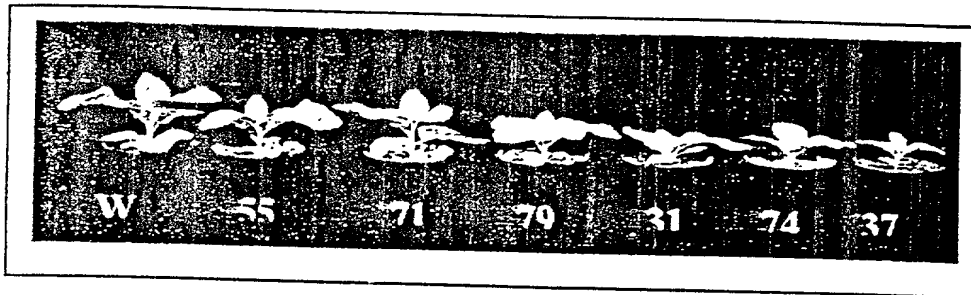
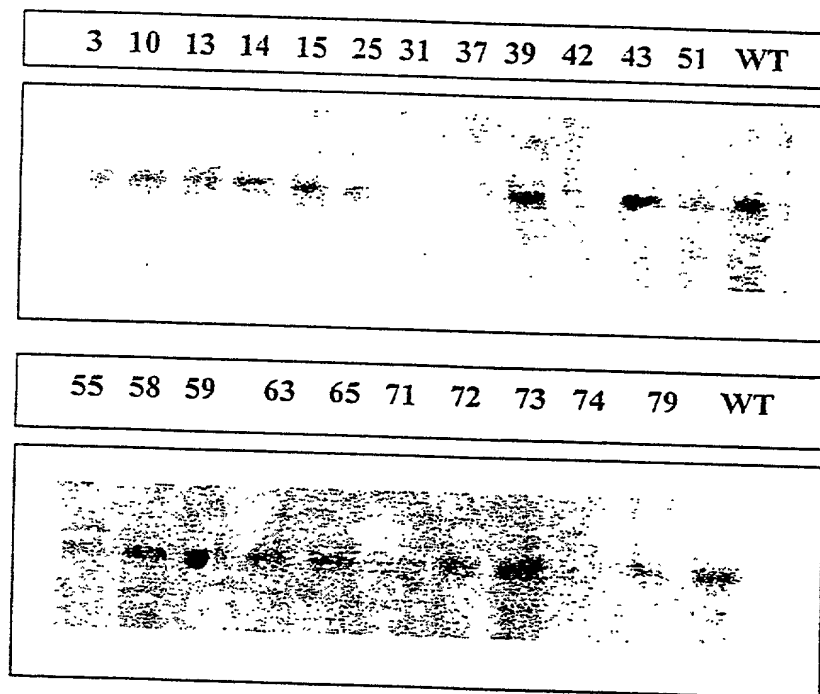


Fig. 4:



# Declaration, Power of Attorney and Petition

Page 1 of 3

0050/050777

Customer No.

We (I), the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

GMP SYNTHETASE FROM PLANTS

the specification of which

☐ is attached hereto.

☐ was filed on \_\_\_\_\_ as

Application Serial No. \_\_\_\_\_

and amended on \_\_\_\_\_.

☒ was filed as PCT international application

Number PCT/EP/00/09245

on 21 September 2000,

and was amended under PCT Article 19

on \_\_\_\_\_ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
19947490.7	Germany	01 October 1999	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

We (I) hereby claim the benefit under Title 35, United States Codes, § 119(e) of any United States provisional application(s) listed below.

_____ (Application Number)	_____ (Filing Date)
_____ (Application Number)	_____ (Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status (pending, patented, abandoned)
_____	_____	_____
_____	_____	_____
_____	_____	_____

And we (I) hereby appoint **Nixon & Vanderhye P.C.**, Attorneys at Law, 1100 North Glebe Road, Arlington, Virginia 22201-4714, our attorneys, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to sign the drawings, to receive the patent, and to transact all business in the Patent Office connected therewith.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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## SEQUENCE LISTING

&lt;110&gt; BASF Aktiengesellschaft

&lt;120&gt; GMP synthetase from plants

&lt;130&gt; DE 19947490.7

&lt;140&gt; 0050-50777

&lt;141&gt; 1999-10-01

&lt;160&gt; 4

&lt;170&gt; PatentIn Vers. 2.0

&lt;210&gt; 1

&lt;211&gt; 1973

&lt;212&gt; DNA

&lt;213&gt; Nicotiana tabacum

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (65)..(1678)

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55

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His Ser Val His Ala Asp Gly Ala Pro Cys Phe Pro Pro Gly Phe Ile

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75

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 Thr Ser Lys Pro Pro Ser Thr Ile Glu Trp Glu  
 530 535

tatatttggt gaccaaagta ggattctttt gtgatttttg gtgcataaca aaaaggaaga 1758

aaatcataat agaaatttag gtccttttgt tatgtggtag aactggttct tgggtaatta 1818

tgtgcaatgc totcaacaat tttgtatggt tatgggtatg atgataccaa attttactca 1878

gatcttggtg gtacattttt cttatccaag tataagtaaca tgtggccagg catcaaaagc 1938

ctattccact caaaaaaaaaa aaaaaaaaaac tcgag 1973

<210> 2

<211> 538

<212> PRT

<213> Nicotiana tabacum

<400> 2

Met Glu Pro Gln Thr Gln Ala Lys Lys Ser Asn Leu Val Leu Ile Leu  
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Asp Tyr Gly Ser Gln Tyr Thr His Leu Ile Thr Arg Arg Ile Arg Ser  
 20 25 30

Leu Ser Ile Phe Ser Leu Thr Ile Asn Gly Thr Ser Ser Leu Asp Ser  
 35 40 45

Ile Lys Glu Leu Asp Pro Arg Val Ile Ile Leu Ser Gly Gly Pro His  
 50 55 60

0050/50777

Ser Val His Ala Asp Gly Ala Pro Cys Phe Pro Pro Gly Phe Ile Glu  
65 70 75 80

Tyr Val Glu Ser Arg Gly Ile His Val Leu Gly Ile Cys Tyr Gly Leu  
85 90 95

Gln Leu Ile Val Gln Lys Leu Gly Gly Val Val Lys Ile Gly Glu Lys  
100 105 110

His Glu Tyr Gly Arg Met Glu Ile Glu Val Gly Lys Asn Val Val Gly  
115 120 125

Gly Leu Phe Gly Asn Thr Glu Ile Gly Asp Lys Gln Val Val Trp Met  
130 135 140

Ser His Gly Asp Glu Ala Val Lys Leu Pro Glu Gly Phe Glu Val Val  
145 150 155 160

Ala Arg Ser Ser Gln Gly Ala Val Ala Ala Ile Glu Asn Arg Glu Arg  
165 170 175

Arg Phe Tyr Gly Leu Gln Tyr His Pro Glu Val Thr His Ser Thr Glu  
180 185 190

Gly Met Arg Thr Leu Arg His Phe Leu Phe Asp Val Cys Gly Val Thr  
195 200 205

Ala Gly Trp Lys Met Glu Asp Val Leu Glu Glu Glu Ile Lys Val Ile  
210 215 220

Lys Gly Met Val Gly Pro Glu Asp His Val Ile Cys Ala Leu Ser Gly  
225 230 235 240

Gly Val Asp Ser Thr Val Ala Ala Lys Leu Val His Lys Ala Ile Gly  
245 250 255

Asp Arg Leu His Cys Val Phe Val Asp Asn Gly Leu Leu Arg Tyr Lys  
260 265 270

Glu Arg Glu Arg Val Met Glu Leu Phe Glu Lys Arg Leu His Leu Pro  
275 280 285

Val Thr Cys Val Asp Ala Thr Glu Glu Phe Leu Ser Lys Leu Lys Gly  
290 295 300

Val Thr Glu Pro Glu Met Lys Arg Lys Ile Ile Gly Lys Glu Phe Ile  
305 310 315 320

Asn Ile Phe Asp Leu Phe Ala His Asp Val Glu Glu Lys Val Gly Lys  
 325 330 335

Lys Pro Ser Tyr Leu Val Gln Gly Thr Leu Tyr Pro Asp Val Ile Glu  
 340 345 350

Ser Cys Pro Pro Pro Gly Ser Gly Arg Thr His Ser His Thr Ile Lys  
 355 360 365

Ser His His Asn Val Gly Gly Leu Pro Lys Asp Met Lys Leu Lys Leu  
 370 375 380

Ile Glu Pro Leu Lys Leu Leu Phe Lys Asp Glu Val Arg Glu Leu Gly  
 385 390 395 400

Lys Ile Leu Asp Ile Ser Glu Asp Phe Leu Lys Arg His Pro Phe Pro  
 405 410 415

Gly Pro Gly Leu Ala Val Arg Ile Pro Gly Asp Val Thr Ala Gly Asn  
 420 425 430

Ser Leu Asp Ile Leu Arg Gln Val Asp Glu Ile Phe Ile Gln Ser Ile  
 435 440 445

Arg Asp Ala Lys Ile Tyr Asp Glu Ile Trp Gln Ala Phe Ala Val Phe  
 450 455 460

Leu Pro Val Lys Thr Val Gly Val Gln Gly Asp Gln Arg Thr His Ser  
 465 470 475 480

His Ala Val Ala Leu Arg Ala Val Thr Ser Gln Asp Gly Met Thr Ala  
 485 490 495

Asp Trp Tyr Tyr Phe Asp Phe Lys Phe Leu Asp Asp Val Ser Arg Lys  
 500 505 510

Ile Cys Asn Ser Val Arg Gly Val Asn Arg Val Leu Leu Asp Ile Thr  
 515 520 525

Ser Lys Pro Pro Ser Thr Ile Glu Trp Glu  
 530 535

<210> 3

<211> 1232

<212> DNA

<213> Physcomitrella patens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (3)..(1148)

&lt;400&gt; 3

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Ile Arg His Glu Ala Thr Ser Thr Gln Gly Asn Ile Ala Ala Ile	
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gaa aat gtg gat tcc aga atc tac gcc ctc caa tac cat ccc gag gtt	95
Glu Asn Val Asp Ser Arg Ile Tyr Ala Leu Gln Tyr His Pro Glu Val	
20 25 30	
acg cac tca gag aaa ggg aca gag act ttg aga cac ttt ttc ctg aat	143
Thr His Ser Glu Lys Gly Thr Glu Thr Leu Arg His Phe Phe Leu Asn	
35 40 45	
gtc tgc ggc atg aag gct gac tgg cag atg cag aat gtg ttg gag gaa	191
Val Cys Gly Met Lys Ala Asp Trp Gln Met Gln Asn Val Leu Glu Glu	
50 55 60	
gag att aaa aag gtc act gcg acc gtc ggc cca gat gat cat gtt att	239
Glu Ile Lys Lys Val Thr Ala Thr Val Gly Pro Asp Asp His Val Ile	
65 70 75	
tgt gca ctc tcc ggg ggc gtg gac tca aca gta gca gct act ctg gtg	287
Cys Ala Leu Ser Gly Gly Val Asp Ser Thr Val Ala Ala Thr Leu Val	
80 85 90 95	
cac cgt gct att gga gat cgc ctt cat tgt gtg ttt gta gat aat ggc	335
His Arg Ala Ile Gly Asp Arg Leu His Cys Val Phe Val Asp Asn Gly	
100 105 110	
ctt tgc aga tac aag gaa aga gaa aga gtg atg gcc aca ttt gtg aaa	383
Leu Cys Arg Tyr Lys Glu Arg Glu Arg Val Met Ala Thr Phe Val Lys	
115 120 125	
gac ctt cat ctg cca gtc act tgt gtg gat gcc act gag cag ttt ctc	431
Asp Leu His Leu Pro Val Thr Cys Val Asp Ala Thr Glu Gln Phe Leu	
130 135 140	
agc aaa ttg aag ggc gtg gta gat cca gag aga aag agg aag atc atc	479
Ser Lys Leu Lys Gly Val Val Asp Pro Glu Arg Lys Arg Lys Ile Ile	
145 150 155	
gga gca gag ttt att gca gtc ttt gat gaa ttt tcg cac aga ttg gag	527
Gly Ala Glu Phe Ile Ala Val Phe Asp Glu Phe Ser His Arg Leu Glu	
160 165 170 175	

aga gag att gga aag atg cct gct ttc ctt gtg cag gga aca ctt tat 575  
 Arg Glu Ile Gly Lys Met Pro Ala Phe Leu Val Gln Gly Thr Leu Tyr  
 180 185 190

cca gat gtc att gag tcg tgt cct cct cca ggg agc ggg aag tcg cat 623  
 Pro Asp Val Ile Glu Ser Cys Pro Pro Pro Gly Ser Gly Lys Ser His  
 195 200 205

tcc cac aca atc aaa agt cat cac aac gtc ggt ggc ttg ccc gag aac 671  
 Ser His Thr Ile Lys Ser His His Asn Val Gly Gly Leu Pro Glu Asn  
 210 215 220

atg aaa ttg aag ttg gtt gag cct ctc aag tgg ctc ttc aaa gac gag 719  
 Met Lys Leu Lys Leu Val Glu Pro Leu Lys Trp Leu Phe Lys Asp Glu  
 225 230 235

gta cgc gaa atg ggt gca ttg ttg gat gta cct gtt tcc ttt ttg aag 767  
 Val Arg Glu Met Gly Ala Leu Leu Asp Val Pro Val Ser Phe Leu Lys  
 240 245 250 255

cgc cat cct ttc cct gga cct gga ttg gcc gtg cga att ctt ggg gat 815  
 Arg His Pro Phe Pro Gly Pro Gly Leu Ala Val Arg Ile Leu Gly Asp  
 260 265 270

gta act cag gac ggc gca ctc gac act atc cgc ttg gtt gat gag atc 863  
 Val Thr Gln Asp Gly Ala Leu Asp Thr Ile Arg Leu Val Asp Glu Ile  
 275 280 285

ttt gtg aac agc att cga gag gca ggt ctt tac gat aag atc tgg cag 911  
 Phe Val Asn Ser Ile Arg Glu Ala Gly Leu Tyr Asp Lys Ile Trp Gln  
 290 295 300

gca ttt gct gtt tat ctg cca gta aag act gtt ggc gtt caa ggc gac 959  
 Ala Phe Ala Val Tyr Leu Pro Val Lys Thr Val Gly Val Gln Gly Asp  
 305 310 315

aaa cgg aca cat tca cac gct gtt gct cta cgt gca att aca agt gaa 1007  
 Lys Arg Thr His Ser His Ala Val Ala Leu Arg Ala Ile Thr Ser Glu  
 320 325 330 335

gac gga atg act gct gac tgg ttt cat ttt gat gga aag ttt ctt gcc 1055  
 Asp Gly Met Thr Ala Asp Trp Phe His Phe Asp Gly Lys Phe Leu Ala  
 340 345 350

gag gta tca tct aaa atc tgc aac agc gta agg ggt atc aat agg gtg 1103  
 Glu Val Ser Ser Lys Ile Cys Asn Ser Val Arg Gly Ile Asn Arg Val  
 355 360 365



gta tac gac att acg tct aaa cct cca tca act gtt gag tgg gaa 1148  
 Val Tyr Asp Ile Thr Ser Lys Pro Pro Ser Thr Val Glu Trp Glu  
 370 375 380

tagacgtcag taatgtatatt tggaagtact gttgggtatg acgattcact gcaataactta 1208

acaaactatt ttataacttca aaaa 1232

<210> 4

<211> 382

<212> PRT

<213> Physcomitrella patens

<400> 4

Ile Arg His Glu Ala Thr Ser Thr Gln Gly Asn Ile Ala Ala Ile Glu  
 1 5 10 15  
 Asn Val Asp Ser Arg Ile Tyr Ala Leu Gln Tyr His Pro Glu Val Thr  
 20 25 30  
 His Ser Glu Lys Gly Thr Glu Thr Leu Arg His Phe Phe Leu Asn Val  
 35 40 45  
 Cys Gly Met Lys Ala Asp Trp Gln Met Gln Asn Val Leu Glu Glu Glu  
 50 55 60  
 Ile Lys Lys Val Thr Ala Thr Val Gly Pro Asp Asp His Val Ile Cys  
 65 70 75 80  
 Ala Leu Ser Gly Gly Val Asp Ser Thr Val Ala Ala Thr Leu Val His  
 85 90 95  
 Arg Ala Ile Gly Asp Arg Leu His Cys Val Phe Val Asp Asn Gly Leu  
 100 105 110  
 Cys Arg Tyr Lys Glu Arg Glu Arg Val Met Ala Thr Phe Val Lys Asp  
 115 120 125  
 Leu His Leu Pro Val Thr Cys Val Asp Ala Thr Glu Gln Phe Leu Ser  
 130 135 140  
 Lys Leu Lys Gly Val Val Asp Pro Glu Arg Lys Arg Lys Ile Ile Gly  
 145 150 155 160  
 Ala Glu Phe Ile Ala Val Phe Asp Glu Phe Ser His Arg Leu Glu Arg  
 165 170 175

Glu Ile Gly Lys Met Pro Ala Phe Leu Val Gln Gly Thr Leu Tyr Pro  
 180 185 190

Asp Val Ile Glu Ser Cys Pro Pro Pro Gly Ser Gly Lys Ser His Ser  
 195 200 205

His Thr Ile Lys Ser His His Asn Val Gly Gly Leu Pro Glu Asn Met  
 210 215 220

Lys Leu Lys Leu Val Glu Pro Leu Lys Trp Leu Phe Lys Asp Glu Val  
 225 230 235 240

Arg Glu Met Gly Ala Leu Leu Asp Val Pro Val Ser Phe Leu Lys Arg  
 245 250 255

His Pro Phe Pro Gly Pro Gly Leu Ala Val Arg Ile Leu Gly Asp Val  
 260 265 270

Thr Gln Asp Gly Ala Leu Asp Thr Ile Arg Leu Val Asp Glu Ile Phe  
 275 280 285

Val Asn Ser Ile Arg Glu Ala Gly Leu Tyr Asp Lys Ile Trp Gln Ala  
 290 295 300

Phe Ala Val Tyr Leu Pro Val Lys Thr Val Gly Val Gln Gly Asp Lys  
 305 310 315 320

Arg Thr His Ser His Ala Val Ala Leu Arg Ala Ile Thr Ser Glu Asp  
 325 330 335

Gly Met Thr Ala Asp Trp Phe His Phe Asp Gly Lys Phe Leu Ala Glu  
 340 345 350

Val Ser Ser Lys Ile Cys Asn Ser Val Arg Gly Ile Asn Arg Val Val  
 355 360 365

Tyr Asp Ile Thr Ser Lys Pro Pro Ser Thr Val Glu Trp Glu  
 370 375 380